Program/Abstract # 175
Loss of primary cilia affect neural crest cell behavior and leads to craniofacial defects
Samantha Brugmann, Nancy C. Allen, Aaron W. James, Zesemayat Mekonnen, Elena Madan, Jill A. Helms
Department of Surgery, Stanford University, Stanford, CA, USA

Craniofacial ciliopathies are disorders of the craniofacial complex caused by disruptions in primary cilia structure and function. To understand the role of primary cilia during craniofacial development we analyzed the behavior of neural crest cells, the progenitors of most of the craniofacial complex, in ciliopathic murine and avian models. We show that loss of primary cilia, via conditional deletion of the intraflagellar transport protein Kif3a, within cranial neural crest cells results in excessive Hedgehog activity and a phenotype that resembles human syndromes with an expanded midline, such as frontonasal dysplasia. In mice, loss of primary cilia did not perturb neural crest cell migration, but did cause aberrant neural crest cell proliferation in a Gli1-dependent manner. The gross phenotypic consequence of this mutation was a dramatic alteration in the pattern of the facial skeleton including a duplicated nasal septum, incomplete growth of the skull, cleft palate, aglossia and micrognathia. Avian Talpid2 embryos exhibit many of the same defects seen in murine and human ciliopathies including facial clefting, aglossia and situs inversus. We found that Talpid2 neural crest cells do not express acetylated tubulin, a marker of the ciliary axoneme or gamma tubulin, a marker of basal bodies. Like their murine counterparts, Talpid2 neural crest cells were able to migrate out of the neural tube into the facial prominences. Taken together, these data provide the first analysis of the role of primary cilia during craniofacial development.

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Program/Abstract # 176
Anterior–posterior patterning of Spemann's organizer by retinoic acid
Abraham Fainsod[a], Michal Gur[a], Hadas Kot-Leibovich[a], Christof Niehrs[b]
[a]*Dept. Dev. Biol. and Cancer Res., IMRIC, Hebrew Univ., Jerusalem, Israel*
[b]*Div. of Molec. Embryol., German Cancer Res. Ctr., Heidelberg, Germany*

Spemann's organizer is the major signaling center regulating the early body plan. At the onset of gastrulation the organizer is patterned along the anterior–posterior (AP) axis. The molecular regulation of this process remains largely uncharacterized. Retinoic acid (RA) signaling becomes active during early gastrula in Spemann's organizer and is required for normal organizer-specific gene expression. RA levels are tightly regulated to control numerous developmental processes, and any deviations result in developmental malformations. In vertebrate embryos, reduction in RA as a result of ethanol exposure induces microcephally and craniofacial malformations strongly suggesting a role in head formation. In our experimental system we knocked down RA signaling which prevented efficient head formation, when reduced either dorsally or in secondary axes, supporting its role in head formation. This effect was strongest when the inhibition of RA biosynthesis was performed around the onset of gastrulation. RA biosynthetic enzymes are expressed in Spemann's organizer, we studied the regulation of their expression and determined the effect of different signaling pathways. Our results suggest that RA biosynthesis is initially activated in the organizer with the onset of gastrulation, and is required for the normal development of the head through regulation of organizer-specific gene expression. A model is proposed where an RA gradient establishes different AP identities, thus regulating the transition from the head to the trunk organizer.

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Program/Abstract # 177
Aquaporin-3b, neural folds and neural tube closure
E. Jean Cornish, Tiffany Hensley, Christa Merzdorf
Dept. of Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA

The aquaporin-3b gene was identified in a screen for direct targets of the Zic1 transcription factor. Compromised zic gene expression causes a variety of defects, among them the neural tube defect holoprosencephaly. We are testing whether aqp-3b may contribute to the mechanisms by which reduced activity of zic genes causes neural tube defects. Expression of the aquaporin-3b gene is limited to the edges of the early neural plate and then to the tips of the forming neural folds during neural tube formation in *Xenopus laevis*. This suggests that Aquaporin-3b (Aqp-3b) protein may have a role in neural tube closure. Aquaporins are transmembrane proteins that form water channels in the plasma membrane and have been shown to facilitate cell movement and cell shape changes, which are both needed for neural tube closure. Our results suggest that aqp-3b morpholino oligonucleotides disrupt normal neural tube closure in Xenopus embryos, indicating that aqp-3b may be required for proper formation of the neural folds. Against our expectations, the actin cytoskeleton does not appear disrupted when aqp-3b translation is inhibited and we are examining this further. Since aqp-3b is an aquaglyceroporin, we are in the process of testing a potential role for glycerol transport during neural tube closure. Very few genes are known to be specifically involved in neural fold formation and we are examining the role of aqp-3b in this process.

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Program/Abstract # 178
Brambleberry mutants reveal new molecular insight into the mechanics of nuclear division during early embryonic development
Elliott W. Abrams, Florence Marlow, Lee Kapp, Tripti Gupta, Mary Mullins
Dept. of Cell and Dev. Biol., Univ. of Penn., Philadelphia, PA, USA

The early cell divisions of animals depend exclusively on maternal gene products supplied to the egg. The earliest embryonic cells are unusual in their large size, which likely require unique cell division mechanisms. Mouse chromokines in KID mutant females produce micronucleated embryos that undergo an early developmental arrest. Using a forward genetic approach in zebrafish, we identified *brambleberry* (*bmb*), a maternal-effect mutant that strikingly resembles the KID phenotype. *bmb* mutants arrest in development shortly after the mid-blastula transition. Examination of the *bmb* nuclear envelope revealed that all cells are micronucleated throughout cleavage. Interestingly, the mitotic spindle appears to function normally during mitosis and is able to attach to wayward chromosomes. Time-lapse imaging of the chromatin combined with EM analysis suggests that during telophase *bmb* chromatin bodies fail to coalesce into a mononucleus and instead persist throughout the ensuing interphase as separate entities, resulting in formation of micronuclei. Positional cloning of the *bmb* gene identified a splice site mutation, which produces a premature stop codon in a novel gene. The conserved wild-type ORF contains two predicted transmembrane